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Enhanced prefrontal serotonin 5-HT$_{1A}$ currents in a mouse model of Williams-Beuren syndrome with low innate anxiety

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Abstract Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder caused by the hemizygous deletion of 28 genes on chromosome 7, including the general transcription factor *GTF2IRD1*. Mice either hemizygously (*Gtf2ird1*$^{+/-}$) or homozygously (*Gtf2ird1*$^{-/-}$) deleted for this transcription factor exhibit low innate anxiety, low aggression and increased social interaction, a phenotype that shares similarities to the high sociability and disinhibition seen in individuals with WBS. Here, we investigated the inhibitory effects of serotonin (5-HT) on the major output neurons of the prefrontal cortex in *Gtf2ird1*$^{-/-}$ mice and their wildtype (WT) siblings. Prefrontal 5-HT receptors are known to modulate anxiety-like behaviors, and the *Gtf2ird1*$^{-/-}$ mice have altered 5-HT metabolism in prefrontal cortex. Using whole cell recording from layer V neurons in acute brain slices of prefrontal cortex, we found that 5-HT elicited significantly larger inhibitory, outward currents in *Gtf2ird1*$^{-/-}$ mice than in WT controls. In both genotypes, these currents were resistant to action potential blockade with TTX and were suppressed by the selective 5-HT$_{1A}$ receptor antagonist WAY-100635, suggesting that they are mediated directly by 5-HT$_{1A}$ receptors on the recorded neurons. Control experiments suggest a degree of layer and receptor specificity in this enhancement since 5-HT$_{1A}$ receptor-mediated responses in layer II/III pyramidal neurons were unchanged as were responses mediated by two other inhibitory receptors in layer V pyramidal neurons. Furthermore, we demonstrate GTF2IRD1 protein expression by neurons in layer V of the prefrontal cortex. Our finding that 5-HT$_{1A}$-mediated responses are selectively enhanced in layer V pyramidal neurons of *Gtf2ird1*$^{-/-}$ mice gives insight into the cellular mechanisms that underlie reduced innate anxiety and increased sociability in these mice, and may be relevant to the low social anxiety and disinhibition in patients with WBS and their sensitivity to serotonergic medicines.

Keywords 5-HT$_{1A}$ receptors · *Gtf2ird1* transcription factor · Williams syndrome · Prefrontal cortex · Social anxiety · Electrophysiology · Mice · Serotonin · GABA-B · Metabotropic glutamate receptors 2/3 (mGluR2/3)

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Introduction

Williams-Beuren syndrome (WBS) is a neurodevelopmental disorder characterized by cardiovascular disease, distinctive facial features, hypersociability, mild to moderate mental retardation and a unique cognitive profile (Bellugi et al. 2000; Mervis et al. 2000; Meyer-Lindenberg et al. 2006). WBS is caused by a common hemizygous deletion of less than 30

genes on chromosome 7, providing the opportunity to study correlations between genes, physiology and social behavior. The identification of individuals with smaller, overlapping deletions has helped geneticists tease apart the contribution of specific genes to the WBS phenotype (Tassabehji et al. 1999; Gagliardi et al. 2003), but since these individuals are rare, this approach has been complemented by the generation of transgenic animal models. Several mouse models have been described in which genes in the WBS critical region have been deleted and these have enabled the assessment of the contribution of individual genes to WBS (Li et al. 1998; Hoogenraad et al. 2002; Meng et al. 2002; Crackower et al. 2003; Young et al. 2008; Osborne 2010).

One of the most striking features of WBS is that of altered social behavior. Despite their heightened friendliness and interest in other people, individuals with WBS encounter problems in daily life because of their inability to interact according to social norms (Gosch and Pankau 1994; Davies et al. 1997; Tager-Flusberg and Sullivan 2000). Until recently, however, no mouse model exhibited behaviors akin to the low social anxiety and disinhibition seen in WBS, making it a difficult aspect of the disorder to study in the laboratory. By running behavioral assays such as the elevated plus maze, open field and resident intruder tests, Young et al. (2008) were able to show that mice with either a reduction or a lack of the general transcription factor gene Gtf2ird1 exhibit decreased anxiety, enhanced sociability and reduced aggression. This study also showed that *Gtf2ird1*$^{-/-}$ mice had normal serotonin (5-HT) levels and significantly elevated 5-hydroxyindoleacetic acid (5-HIAA) levels in prefrontal cortex (Young et al. 2008).

It has been suggested that dysregulation of prefrontal output in individuals with WBS accounts for the reduced social anxiety in this population (Meyer-Lindenberg et al. 2005). The prefrontal cortex plays a key role in anxiety (Chua et al. 1999; Davidson et al. 1999; Liotti et al. 2000; Osuch et al. 2000) and its neuromodulation by 5-HT is important in mediating this function. In particular, imaging studies in humans indicate that reduced 5-HT$_{1A}$ receptor binding correlates with trait anxiety both in healthy volunteers (Tauscher et al. 2001) and in patients afflicted with social anxiety disorder (Lanzenberger et al. 2007). In mice, 5-HT$_{1A}$ receptor knockouts exhibit increased anxiety (Heisler et al. 1998; Parks et al. 1998; Ramboz et al. 1998) and expression of this receptor in forebrain structures appears to be sufficient to establish normal anxiety responses (Gross et al. 2002). In contrast, overexpression of 5-HT$_{1A}$ receptors leads to decreased anxiety (Kusserow et al. 2004).

While it is known that *Gtf2ird1*$^{-/-}$ mice exhibit low anxiety, reduced aggression and increased sociability (Young et al. 2008), it is not known how prefrontal neurons in these mice respond to 5-HT. Here, we use whole cell electrophysiology in acute brain slices to examine how the major output neurons of prefrontal cortex are modulated by serotonin in *Gtf2ird1*$^{-/-}$ mice and their wildtype littermate controls. Since significant differences emerged on this measure between the genotypes, we tested the specificity of these differences for the cortical layer and for the receptors involved. We also investigated the expression of genes that may affect 5-HT$_{1A}$ receptor function and correlated the localization of GTF2IRD1 protein with the neurophysiological difference found in the *Gtf2ird1*$^{-/-}$ mice. This is the first examination of cellular neurophysiology in a mouse model of WBS exhibiting a low anxiety phenotype.

Materials and methods

Gtf2ird1$^{-/-}$ mice

Gtf2ird1 knockout mice were generated by homologous recombination as described previously (Young et al. 2008). The mice were maintained on the same CD1 background as they were for the previous behavioural analyses (Young et al. 2008) and had reached the N8 generation of backcrossing at the time of these experiments. For all experiments *Gtf2ird1*$^{-/-}$ mice and wildtype (WT) littermates were generated through the intercrossing of *Gtf2ird1*$^{+/-}$ heterozygous mice.

Brain slice preparation

Coronal slices (400 μm thick) of the medial prefrontal cortex were prepared from male, adult *Gtf2ird1*$^{-/-}$ mice and their WT littermates. The brain was cooled as rapidly as possible with 4°C oxygenated sucrose artificial cerebrospinal fluid (ACSF) (254 mM sucrose was substituted for NaCl). Prefrontal slices were cut from anterior to posterior using the appearance of white matter and the corpus callosum as anterior and posterior guides. The slices were cut on a Dosaka Linear Slicer (SciMedia), and were transferred to 30°C oxygenated ACSF (containing the following, in mM: 126 NaCl, 10 D-glucose, 24 $NaHCO_3$, 2 $CaCl_2$, 2 $MgSO_4$, 3 KCl, 1.25 NaH_2PO_4, pH 7.4) in a prechamber (Automate Scientific) and allowed to recover for at least 1.5 h before the beginning of an experiment. For whole recordings, slices were placed in a modified chamber (Warner Instruments) mounted on the stage of an Olympus BX50WI microscope. Regular ACSF was bubbled with 95% oxygen and 5% carbon dioxide and flowed over the slice at 30°C with a rate of 3–4 ml/min.

Electrophysiology

Whole-cell patch electrodes (2–3 MΩ) contained the following (in mM): 120 K-gluconate, 5 KCl, 2 MgCl, 4 K-ATP, 0.4 Na_2-GTP, 10 Na_2-phosphocreatine, and 10 HEPES buffer (adjusted to pH 7.33 with KOH). Layer V pyramidal neurons were patched under visual control using infrared differential interference contrast microscopy in the cingulate and prelimbic regions, as illustrated in the schematic in Fig. 1a. In voltage-clamp, neurons were held at −75 mV, near the calculated equilibrium potential for chloride under these conditions, and currents were recorded using continuous single electrode voltage-clamp mode with a Multiclamp 700b (Molecular Devices), acquired and low-pass filtered at 3 kHz with pClamp10.2/Digidata1440 (Molecular Devices). The reversal potential for K^+ is −97 mV under these recording conditions. After gaining access to a cell, membrane potential, spike amplitude and input resistance were measured in current clamp. Preliminary concentration response experiments in voltage-clamp (5-HT; 3 μM–100 μM, 30 s) showed that 5-HT elicited outwards currents in layer V pyramidal neurons, consistent with previous work in rats (Béïque et al. 2004). Serotonin (30 μM), $GABA_B$ agonist baclofen (30 μM) and $mGluR_{2/3}$ agonist APDC (30 μM) all elicited near-maximal responses that could be replicated with a second application after 5–7 min of washout. Currents were measured in Clampfit by subtracting the mean outward current at the peak of the response from the mean holding current at baseline.

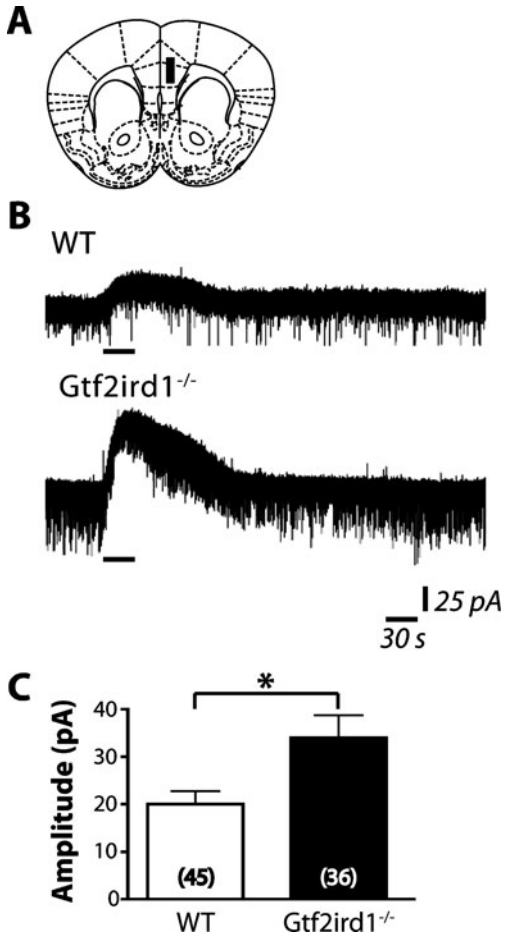

Fig. 1 Serotonin (5-HT) elicits a larger inhibitory outward current in prefrontal layer V pyramidal neurons from *Gtf2ird1*$^{-/-}$ mice when compared to recordings from wildtype (WT) mice. **a** Schematic of a coronal section of prefrontal cortex. The vertical bar indicates the region in which whole-cell recordings of layer V pyramidal neurons were performed. **b** Representative voltage-clamp traces show that 5-HT application induces a larger inhibitory outward current in *Gtf2ird1*$^{-/-}$ than WT, as measured at its peak. Gray bar denotes 5-HT application. **c** Bar graph of average peak amplitudes of outward currents. *N* values are bracketed; error bars represent the standard error and highlight the variance of the current distributions (* denotes $P<0.05$)

Drugs

t-APDC and baclofen were obtained from Tocris bioscience (Burlington, ON, Canada), serotonin creatinine sulfate and WAY-100635 from Sigma-Aldrich (St-Louis, MO, USA) and TTX from Alomone labs Ltd. (Jerusalem, Israel). All drugs were bath applied.

Statistical analysis

We analyzed normality, conducted one-way analyses of variance, and computed means and moments of peak electrophysiological currents with JMP software (a SAS software, Cary, NC). Significance level was set at 0.05.

Quantitative RT-PCR

Expression analysis was carried out using total RNA extracted from dissected adult frontal cortex with TriReagent (Sigma-Aldrich Canada, Oakville, ON). Following DNase treatment (Turbo DNA free, Ambion), 5 μg of RNA was converted to cDNA using the SuperScript™ First-Strand Synthesis System (Invitrogen Canada Inc., Burlington, ON) and random hexamer primers. Samples were diluted 1/100 with sterile water and used directly in real-time assays using the Power SYBR Green PCR Master mix and ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster City, CA) as described previously (Somerville et al. 2005). All samples were run in triplicate and the experiment was repeated twice with consistent results. Absolute quantification analysis, normalized to the expression of the control gene succinate dehydrogenase (Sdha), was used to determine expression levels. Pair wise comparison of gene expression in *Gtf2ird1*$^{-/-}$ (n=12) and WT (n=12) mice was performed using a two-tailed Student's *t*-test in PAST (PAlaeontological STatistics)(http://folk.uio.no/ohammer/past/). Primer sequences are as follows: 5-ht1aF 5′-CTGGGGACGCTCATT

TTCT-3′; 5-ht1aR 5′-CCAAGGAGCCGATGAGATAG-3’; 5-ht1bF 5’-GAGTCCGGGTCTCCTGTGTA-3’; 5-ht1bR 5’-TAGC GGCCATGAGTTTCTTC-3’; 5-ht2aF 5’-TGTGCCG TCTGGATTTACCT-3’; 5-ht2aR 5’-TGAATGGGGT TCTGGATAGC-3’; 5-ht2cF 5’-CATGGCAGTAAGCAT GGAGA-3’ ; 5-ht2cR 5’-AGTCCCACCAGCATATC AG C-3’; MaoaF 5’-GTGCCTGGTCTGCTCAAGAT-3’ ; MaoaR 5’-TTCAGGACTGGGGCTGTTTA-3’; SdhaF 5’-TGATCTTCGCTGGTGTGGATGTCA-3’; SdhaR 5’-CCC ACCCATGTTGTAATGCACAGT-.

Generation of *Gtf2ird1*$^{(XS0608)Wtsi}$ mice

Clone XS0608 (embryonic stem cells 129SvEv) carrying an insertion of the gene trap vector pGT0lxf in intron 4 of the Gtf2ird1 gene (*Gtf2ird1*$^{(XS0608)Wtsi}$) (available from the Sanger Institute Gene Trap Resource) was used to generate mutant mice after injection into C57BL/6 blastocysts. The resulting chimeras were bred to CD1 females to produce *Gtf2ird1*$^{(XS0608)Wtsi}$ mice that express a GTF2IRD1-LacZ fusion protein under the control of the endogenous *Gtf2ird1* promoter. Mice were genotyped using the primer mix: gtIRD1i4F 5'-CCCACCGACCTTA TCTGAAC-3'; gtEn2i1R 5'-GGGTCTCTTTGTCA GGGTCA-3'. The size of the amplicon is 466 bp for the *Gtf2ird1*$^{(XS0608)Wtsi}$ mutant allele.

X-gal staining of the *Gtf2ird1*$^{(XS0608)Wtsi}$ mouse cortex

Male adult WT and *Gtf2ird1*$^{(XS0608)Wtsi}$ mice were perfused with PBS containing 2 mM $MgCl_2$ (PBS+Mg) followed by freshly made 2% PFA/0.2% glutaraldehyde in PBS+Mg. Whole brains were frozen in isopentane on dry ice and 50 μm coronal sections were cut on a cryostat. Free floating sections were rinsed in PBS+Mg then fixed for 10 min in 2% PFA/0.2%glutaraldehyde in PBS+Mg. Sections were rinsed in PBS+Mg several times and immersed in LacZ staining solution overnight containing: 5 mM Potassium ferricyanide, 5 mM potassium ferrocyanide, 0.01% sodium deoxycolate/0.02% NP40, 1 mg/ml *X-Gal* in PBS+Mg. Sections were washed with PBS+Mg, transferred to slides, counterstained with Eosin Y, dehydrated, and mounted in Cytoseal. Sections were viewed on a Leica DMRBE microscope (Leica Microsystems, Germany) using PL Fluotar 5X and 20X objectives.

Results

Serotonin elicits larger outward currents in layer V pyramidal neurons in *Gtf2ird1*$^{-/-}$ mice

The initial study was performed blind to genotype in a group of 5 *Gtf2ird1*$^{-/-}$ and 5 WT animals. In both groups, bath application of 5-HT (30 μM, 30 s) induced prominent inhibitory outward currents in voltage-clamp that were repeatable on a second application after washout. In the blind experiments, the mean outward current was significantly larger in the *Gtf2ird1*$^{-/-}$ mice (controls: 18±3.4 pA, n=31; *Gtf2ird1*$^{-/-}$ mice 38±6.4 pA, n=26; two-tailed unpaired t test, P<0.05). Example recordings are shown in Fig. 1 together with the mean 5-HT-elicited currents from a larger dataset, which combines the blind recordings with additional neurons recorded in brain slices from each genotype for subsequent pharmacological experiments (controls: 20 ± 2.8 pA, n=45; *Gtf2ird1*$^{-/-}$ mice: 34±4.7 pA, n=36; two-tailed unpaired t test, P<0.05). Of note, the genotype difference in the modulatory effect of 5-HT occurred in the absence of significant differences in the membrane properties of the neurons: the average membrane potential was −84±1 mV in controls and −83±2 mV in *Gtf2ird1*$^{-/-}$ mice; input resistance was 140±20 MΩ in controls and 132±14 MΩ in *Gtf2ird1*$^{-/-}$; and spike amplitude was 77±3 mV in controls and 79±2 mV in *Gtf2ird1*$^{-/-}$ mice. Thus, despite similar baseline properties, 5-HT elicits a greater inhibitory outward current in layer V output neurons of prefrontal cortex in *Gtf2ird1*$^{-/-}$ mice than in WT controls.

In both genotypes, the 5-HT-elicted outward currents are direct and mediated by 5-HT_{1A} receptors

We next examined whether the observed currents were directly mediated by 5-HT receptors located on the recorded cell. In order to answer this question, we applied 5-HT before and after blocking the voltage-gated sodium channels necessary for action potential dependent neurotransmitter release with tetrodotoxin (TTX, 2 μM, 10 min). The 5-HT-elicited outward currents were resistant to TTX, with current amplitudes that were 99±14% (n=5) that of currents recorded prior to TTX application in controls and 98±10% (n=4) in *Gtf2ird1*$^{-/-}$ mice (Suppl. Figure 1). Paired t tests revealed that responses were not significantly different before and after TTX application in WT (P=NS) or *Gtf2ird1*$^{-/-}$ animals (P=NS). These results suggest that the recorded responses are directly mediated by 5-HT receptors on the layer V neurons.

Of the many subtypes of 5-HT receptors (Nichols and Nichols 2008), the 5-HT_{1A} receptor subtype is prominently expressed in the prefrontal cortex where it mediates well-documented inhibitory influences (Araneda and Andrade 1991; Béïque et al. 2004; Goodfellow et al. 2009). Further, prior studies have highlighted the importance of this receptor in prefrontal cortex for regulating anxiety (Tauscher et al. 2001; Gross et al. 2002; Lanzenberger et al. 2007). To test whether the observed 5-HT responses were indeed mediated by the 5-HT_{1A} receptor subtype, we

applied the selective 5-HT_{1A} antagonist WAY-100635 (30 nM, 10 min). As shown in Fig. 2, the responses to 5-HT (30 μM, 30 s) after WAY-100635 (30 nM, 10 min) were almost completely eliminated: 100±0% (n=4) suppression in controls and 99±8% (n=7) suppression in *Gtf2ird1*$^{-/-}$ mice. In both genotypes, matched pair t-test statistics confirm a significant current reduction in the presence of WAY-100635 (P<0.05). In layer V pyramidal cells of both controls and *Gtf2ird1*$^{-/-}$ animals, therefore, the recorded 5-HT responses appear to be directly mediated by 5-HT_{1A} receptors.

Serotonin 5-HT_{1A} outward currents are unchanged in prefrontal layer II/III

Since 5-HT has also been shown to exert inhibitory influences on layer II/III cells of the medial prefrontal cortex through 5-HT_{1A} receptors (Goodfellow et al. 2009), we examined whether these outward currents were enhanced the *Gtf2ird1*$^{-/-}$ mice. However, the 5-HT responses recorded in layer II/III cells were not significantly different between *Gtf2ird1*$^{-/-}$ and WT animals (Suppl. Figure 2). Following bath application of 5-HT (30 μM, 30 s), the average inhibitory current recorded in cells from WT animals was 26±3.2 pA (n=12 cells) and 29±12 pA in the *Gtf2ird1*$^{-/-}$ (n=9 cells) (P=NS). Thus, 5-HT specifically elicits larger inhibitory responses in *Gtf2ird1*$^{-/-}$ mice in layer V and not in layer II/III.

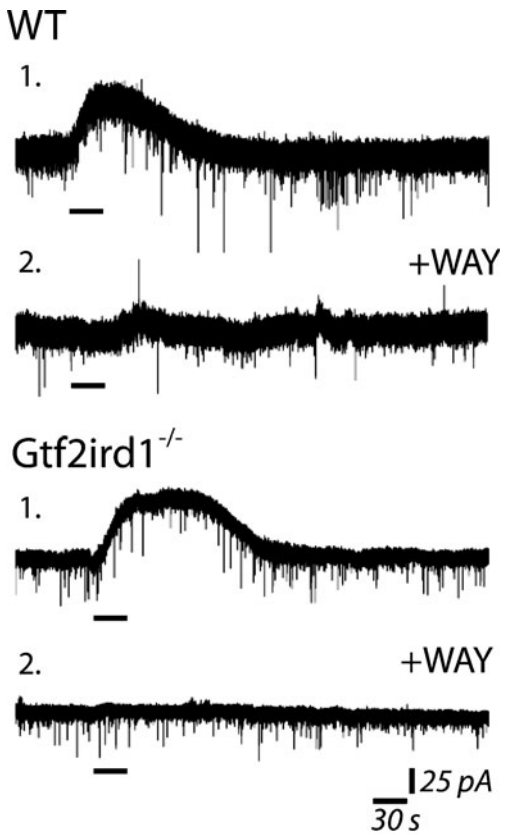

Fig. 2 Serotonergic currents in layer V pyramidal neurons mediated by 5-HT_{1A} receptors in wildtype (WT) and *Gtf2ird1*$^{-/-}$ mice. In neurons from each genotype, *(1.)* the 5-HT-elicited outward currents were suppressed by *(2.)* the selective antagonist of 5-HT_{1A} receptors, WAY-100635 (30 nM, 10 min). Gray bar indicates 5-HT application. All the above traces were recorded after application of TTX (2 μM, 10 min), indicating that the 5-HT outward currents were mediated directly by 5-HT_{1A} receptors located on the recorded cells

Other inhibitory currents are not enhanced in layer V of the *Gtf2ird1*$^{-/-}$ mice

If the downstream effectors of 5-HT_{1A} receptors are altered in *Gtf2ird1*$^{-/-}$ mice, then the currents elicited by other $G_{i/o}$-coupled receptors might also be enhanced. We therefore investigated the outward currents elicited by two other inhibitory $G_{i/o}$-coupled receptors, the $mGluR_{2/3}$ and $GABA_B$ receptors, in layer V pyramidal neurons of the *Gtf2ird1*$^{-/-}$ mice. These neurons are known to also express $mGluR_{2/3}$ (Petralia et al. 1996; Melendez et al. 2004) and $GABA_B$ receptors (Charles et al. 2003). The $mGluR_{2/3}$ agonist t-APDC (30 μM, 30 s) and the $GABA_B$ agonist baclofen (3 μM, 15 s) were bath applied and induced outward currents similar to those elicited by 5-HT in layer V pyramidal cells. As illustrated in Fig. 3, the responses to both agonists were however not significantly different between genotypes. The $mGluR_{2/3}$ agonist t-APDC elicited inhibitory outward currents of 21±6.0 pA in WT (n=6) and 24±7.0 pA in *Gtf2ird1*$^{-/-}$ mice (n=4) (P=NS) while baclofen induced currents of 55±5.8 pA in controls (n=6) and 66±8.5 pA in *Gtf2ird1*$^{-/-}$ (n=8) (P=NS). These data suggest that altered receptor function in the *Gtf2ird1*$^{-/-}$ mice may be specific to 5-HT_{1A} receptors since currents elicited by two other $G_{i/o}$ receptors in layer V pyramidal neurons are not similarly affected.

Unchanged expression of 5-HT-related genes in frontal cortex

We examined the expression of several 5-HT receptors and the enzyme monoamine oxidase (MAO-A) in frontal cortex of *Gtf2ird1*$^{-/-}$ mice using quantitative real-time PCR. RNA levels did not differ significantly between *Gtf2ird1*$^{-/-}$ and WT mice for any of the genes tested (*Htr1a; Htr1b; Htr2a; Htr2c; Maoa*; unpaired t tests, P=NS, Table 1).

GTF2IRD1 protein expression is predominantly in layer V of the frontal cortex

We used *Gtf2ird1*$^{(XS0608)Wtsi}$ mice to examine GTF2IRD1 expression in the adult prefrontal cortex. In coronal sections from a similar location to that used for the electrophysiological recordings, there is obvious somatic expression of the GTF2IRD1-LacZ fusion protein in many large neurons

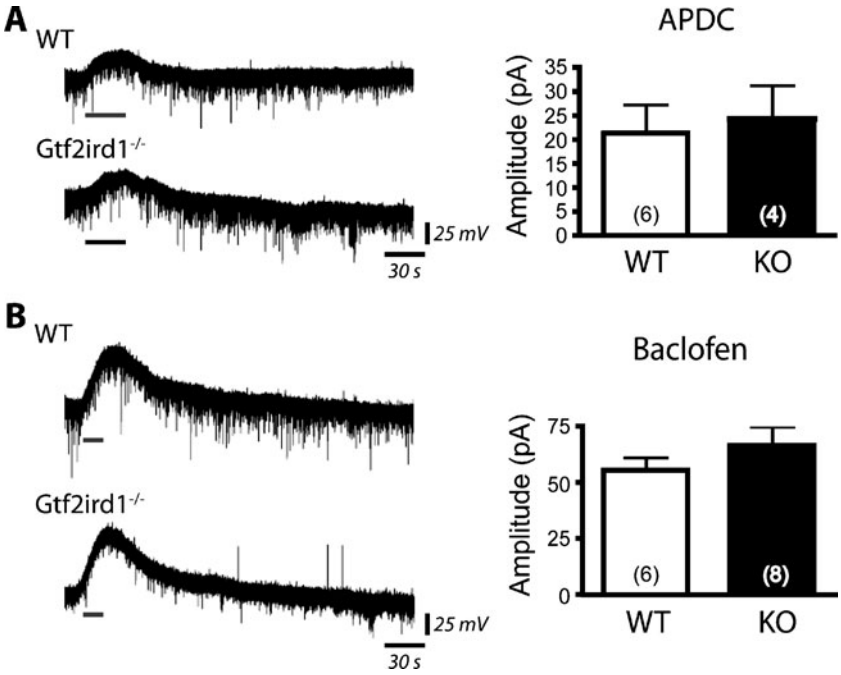

Fig. 3 Stimulation of two other $G_{i/o}$-coupled receptors implicated in anxiety produces similar responses in layer V pyramidal neurons in both wildtype (WT) and *Gtf2ird1*$^{-/-}$ mice. **a** The $mGluR_{2/3}$ agonist *t*-APDC (30 μM, 30 s) induces similar responses in both genotypes (WT: 21±6 pA; *Gtf2ird1*$^{-/-}$ mice: 24±7 pA; *P*=NS). Gray bars denote *t*-APDC application. **b** Moreover, the $GABA_B$ agonist baclofen (3 μM, 15 s) elicits similar responses in both genotypes (WT: 55±6 pA; *Gtf2ird1*$^{-/-}$ mice: 66±9 pA; *P*=NS). Gray bars indicate baclofen application

in layer V, as illustrated in Fig. 4. Layer V pyramidal neurons are large and widely-spaced in contrast with the smaller, more densely-packed neurons of layer II/III. Some diffuse LacZ staining could also be seen in layer II/III, as well as in layer I. It should be noted that the apical dendrites of layer V pyramidal cells extend to the pial surface and arborize extensively in layer II/III.

Discussion

In this study, we investigated how pyramidal cells in prefrontal cortex are modulated by 5-HT in mice lacking the general transcription factor gene Gtf2ird1. These *Gtf2ird1*$^{-/-}$ mice have previously been shown to exhibit a low anxiety, low aggression and high sociability phenotype (Young et al. 2008), which is reminiscent of symptoms seen in individuals with WBS. We show that 5-HT elicits enhanced outward currents in layer V pyramidal cells of the prefrontal cortex via postsynaptic 5-HT_{1A} receptors in *Gtf2ird1*$^{-/-}$ mice compared to their WT littermates. This enhancement of an inhibitory current appears somewhat layer and receptor specific. Consistent with the expression pattern of GTF2IRD1 protein, serotonin 5-HT_{1A} outward currents are not enhanced in layer II/III pyramidal neurons of *Gtf2ird1*$^{-/-}$ mice. In addition, inhibitory currents mediated by other $G_{i/o}$-coupled $mGluR_{2/3}$ and $GABA_B$ receptors in layer V pyramidal neurons are unchanged. Together, our data raise important questions about the mechanism that underlies the enhanced 5-HT_{1A} currents in layer V and the consequences of this current for prefrontal functional connectivity in this mouse characterized by a low anxiety phenotype.

Implications of elevated 5-HT_{1A} currents for brain function in a low anxiety mouse phenotype

Evidence suggests that elevated 5-HT_{1A} receptor function is inversely correlated with anxiety. In rodents, 5-HT_{1A} receptor knockout mice have been shown to exhibit higher anxiety (Heisler et al. 1998; Parks et al. 1998; Ramboz et al. 1998) while overexpression of 5-HT_{1A} receptors leads

Table 1 Expression levels of 5-HT-related genes in the mouse frontal cortex

Expression Level	Htr1a		Htr1b		Htr2a		Htr2c		Maoa	
	WT	Gtf2ird1$^{-/-}$	WT	Gtf2ird1$^{-/-}$	WT	Gtf2ird1$^{-/-}$	WT	Gtf2ird1$^{-/-}$	WT	Gtf2ird1$^{-/-}$
Mean (*n*=12)	0.0590	0.0666	0.0957	0.0914	0.5541	0.6194	0.2478	0.2697	0.1649	0.1509
SD	0.0124	0.0187	0.0342	0.0332	0.1859	0.1030	0.0683	0.0273	0.0503	0.0166
SEM	0.0036	0.0054	0.0099	0.0096	0.0537	0.0297	0.0197	0.0079	0.0145	0.0048
95% CI	0.0070	0.0106	0.0193	0.0188	0.1052	0.0583	0.0386	0.0155	0.0285	0.0094

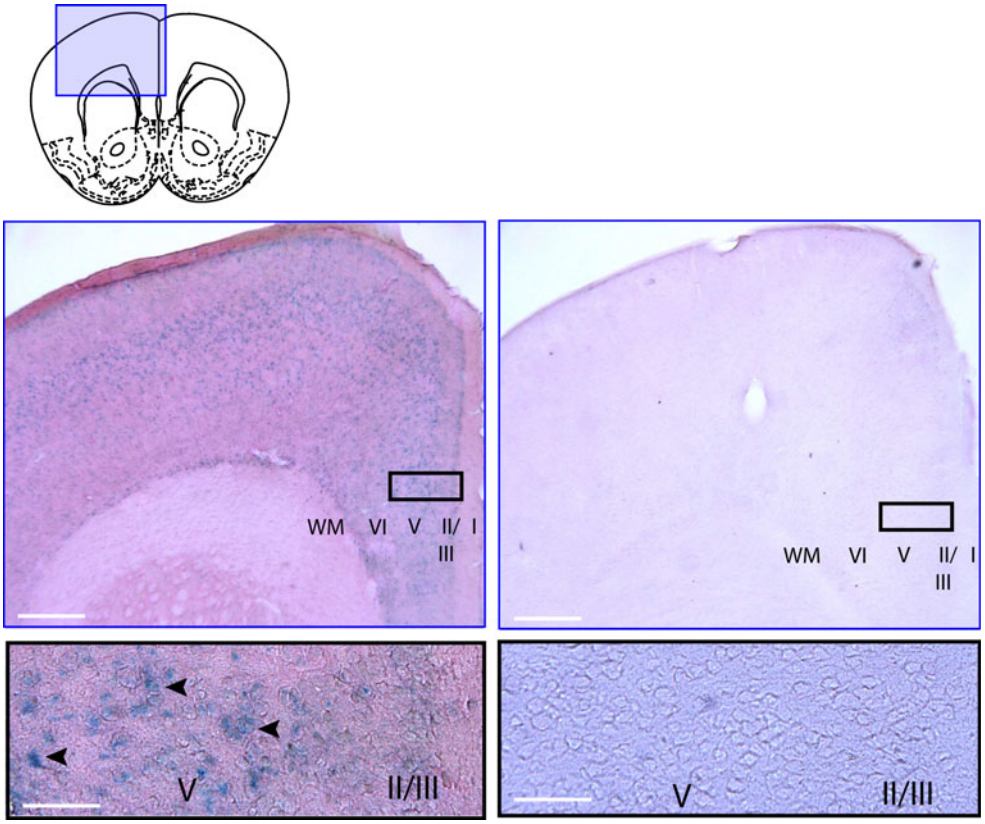

Fig. 4 The transcription factor GTF2IRD1 is expressed in layer V neurons of the prefrontal cortex. Top, the blue rectangle on the schematic depicts the region of prefrontal cortex shown in the images below. Coronal sections of prefrontal cortex from an adult *Gtf2ird1*$^{(XS0608)Wtsi}$ mouse (*left*) and a control *Gtf2ird1*$^{+/+}$ mouse (*right*) stained for LacZ activity. WM denotes white matter, and cortical layers are indicated in roman numerals. Scale bar: 300 μm. Higher magnification images illustrate the laminar pattern of staining in medial prefrontal cortex from the regions shown above in black rectangles. Note the somatic expression of the GTF2IRD1-LacZ fusion protein in layer V neurons (examples shown with arrowheads). Scale bar: 50 μm

to decreased anxiety (Kusserow et al. 2004). The inhibitory effects of 5-HT on layer V pyramidal neurons may have profound effects on brain function since these cells are considered the primary output neurons of the prefrontal cortex. They send projections to the amygdala, hypothalamus, and striatum; in addition, they are the only source of cortical feedback to several key neuromodulatory nuclei. For example, the dorsal raphe nucleus receives its only cortical projection from layer V pyramidal cells of the prefrontal cortex (Peyron et al. 1998; Vertes 2004; Gabbott et al. 2005; Gonçalves et al. 2009). Because these cells provide 5-HT neurons of the raphe with negative feedback (Hajós et al. 1998; Celada et al. 2001; Jankowski and Sesack 2004), increased inhibition of layer V neurons would disinhibit the raphe and thereby increase 5-HT release in the prefrontal cortex. Previous HPLC findings in the *Gtf2ird1*$^{-/-}$ mice indicate that the levels of the 5-HT metabolite 5-HIAA are significantly increased in the prefrontal cortex and amygdala of *Gtf2ird1* knockout mice while 5-HT levels remain unchanged (Young et al. 2008). Increases in the ratio of 5-HIAA to 5-HT are thought to be an indicator of 5-HT turnover (Tozer et al. 1966). Here, we report that levels of mRNA for MAO-A are not significantly different in *Gtf2ird1*$^{-/-}$ mice. Together, these findings support the hypothesis that 5-HT release is enhanced in the prefrontal cortex of these mice.

The prefrontal cortex also shares dense and reciprocal connections with the amygdala (Ghashghaei and Barbas 2002; Gabbott et al. 2005). Interactions between the prefrontal cortex and the amygdala are of great interest in the investigation of neural mechanisms underlying the WBS phenotype because of their role in anxiety and social cognition. Neuroimaging studies have implicated the prefrontal cortex in anxiety both in healthy controls (Chua et al. 1999; Liotti et al. 2000; Tillfors et al. 2001) and in subjects with affective disorders (Osuch et al. 2000; Tillfors et al. 2001; Lanzenberger et al. 2007). Further, interactions of the prefrontal cortex and amygdala have been shown to play a critical role in social cognition (Morgan et al. 1993; Prather et al. 2001; Amaral 2002; Quirk et al. 2003). Recently, it has been confirmed that prefrontal modulation of the amygdala is dysregulated in WBS (Meyer-Lindenberg et al. 2005) but the cellular mechanisms underlying this phenomenon remain to be elucidated. We can posit in light of our findings that greater inhibitory influences exerted by 5-HT on prefrontal output cells may contribute to this functional uncoupling.

Possible mechanisms of altered 5-HT$_{1A}$ receptor function in *Gtf2ird1*$^{-/-}$ mice

There have been very few targets of the GTF2IRD1 transcription factor identified (O'Mahoney et al. 1998;

Polly et al. 2003; Jackson et al. 2005). While putative DNA binding sequences have been found (Thompson et al. 2007; Lazebnik et al. 2008; Palmer et al., 2010) none of the genes evaluated in this study were determined to contain known GTF2IRD1 binding sequences, consistent with the lack of altered expression in the tested 5-HT receptors and MAO-A genes. Since *Gtf2ird1* is expressed widely during embryogenesis (Palmer et al. 2007), the elevated 5-HT_{1A} currents in layer V of prefrontal cortex could be an indirect effect of constitutively deleting this transcription factor. However, our finding that GTF2IRD1-LacZ fusion protein is expressed in layer V pyramidal neurons of the adult cortex suggests that direct effects may also result from loss of *Gtf2ird1* transcriptional control.

The functional differences in 5-HT_{1A} outward currents we observed in layer V pyramidal neurons could arise at several potential levels. We suggest that the enhanced inhibitory currents observed in the *Gtf2ird1*$^{-/-}$ mice do not result from enhanced expression of the receptor since we observed no difference in prefrontal 5-HT_{1A} receptor mRNA. The inhibitory actions of 5-HT_{1A} receptors are mainly mediated by increasing potassium conductance via G-protein linked inwardly rectifying potassium (GIRK) channel activation (Innis et al. 1988; Williams et al. 1988; Penington et al. 1993). Since enhanced 5-HT_{1A}-mediated responses could be due to downstream changes from the 5-HT_{1A} receptor, such as its G-protein linked potassium channel activation, we investigated the currents mediated by two other inhibitory $G_{i/o}$-coupled receptors, the group II metabotropic glutamate ($mGluR_{2/3}$) and $GABA_B$ receptors. Because these receptors are also linked to G-protein activated potassium channels (Andrade et al. 1986; Innis et al. 1988), we postulated that if responses mediated by these receptors were also enhanced, altered function of downstream effectors shared by all three receptors was likely at play in mediating these larger currents. This, however, was not the case, suggesting that the mechanisms underlying increased 5-HT_{1A}-mediated inhibitory responses are receptor specific.

In this regard, aberrant 5-HT_{1A} receptor function could arise from altered sensitivity to the systemic stress response. The hypothalamic-pituitary-adrenal (HPA) axis plays a crucial role in mediating anxiety behaviors and has been shown to interact with the serotonergic neuromodulatory system (Lanfumey et al. 2008). Exposure to stressors leads to the release of corticosteroids from the adrenal gland in response to hormonal signals from the hypothalamus and pituitary gland. Interestingly, chronic corticosteroid exposure has been shown to significantly reduce 5-HT_{1A}-mediated inhibitory currents in the brain whereas adrenalectomy has the opposite effect. Furthermore, it has been suggested that this modulation of postsynaptic 5-HT_{1A} function by corticosteroids can be mediated at the level of G protein coupling (Okuhara and Beck 1998). In light of this prior study, we can speculate that the low anxiety *Gtf2ird1*$^{-/-}$ mice may be exposed to less circulating corticosteroids or that they could be less sensitive to these hormones. The integrity of the HPA axis in these mice is a critical subject for future study.

Relevance of the *Gtf2ird1*$^{-/-}$ mouse model to WBS

Individuals with WBS have been said to be gregarious, overly friendly and to possess a heightened interest in other people (Bellugi et al. 1999; Jones et al. 2000; Doyle et al. 2004). In this respect, the finding that *Gtf2ird1*$^{-/-}$ mice exhibit low anxiety and increased interest in other mice proves to be highly valuable, especially in light of the fact that prior to the characterization of this mouse, no other mouse model exhibited behaviors akin to the hypersociability seen in WBS (Young et al. 2008). The caveat is, however, that *Gtf2ird1*$^{-/-}$ mice exhibit decreased anxiety on tasks of both social and non-social nature, as on the resident intruder test and elevated plus maze task, respectively. This stands in contrast to individuals with WBS, who despite their low social anxiety tend to display a high degree of non-social anxiety. Indeed, approximately half of patients have specific phobias (Dykens 2003; Leyfer et al. 2006). It has not been examined, however, whether the *Gtf2ird1*$^{-/-}$ mice may show enhanced anxiety under certain conditioning paradigms despite their seemingly low innate anxiety.

Interestingly, anecdotal evidence suggests that people with WBS are hypersensitive to the most commonly prescribed treatment for non-social anxiety, the selective serotonin reuptake inhibitors (SSRIs) (Cherniske et al. 2004; Pober 2006). Standard doses of these medicines are associated with adverse effects such as further disinhibition (Cherniske et al. 2004). Chronic administration of such medicines is well-known to downregulate 5-HT_{1A} autoreceptors within the dorsal raphe, but has only recently been shown to have the opposite effect, a pronounced enhancement, on the function of prefrontal 5-HT_{1A} receptors (Moulin-Sallanon et al. 2009). Our results give insight into a prefrontal cellular mechanism that would contribute to the hypersensitivity of individuals with WBS to SSRIs.

Conclusions

The *Gtf2ird1*$^{-/-}$ mouse model allows us to study how the deletion of one of the genes in the WBS critical region on chromosome 7 can alter neurophysiology and lead to the enhancement of inhibitory 5-HT currents in the main output neurons of the prefrontal cortex. As such, this model can help guide future pharmacological and functional human imaging studies in WBS and provide insight into alternative therapeutic targets to help restore normal prefrontal excitability.

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